Maximising establishment success of *Amphibolis antarctica* seedlings

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Abstract

Coastal developments of all scales impact on seagrass meadows. Mitigation for loss of seagrass ecosystems is increasingly being required by regulatory agencies to compensate for coastal developments and while many attempts have been made to transplant seagrasses, there are concerns about the long-term viability of these efforts, as well as the sustainability of harvesting natural seagrass material for transplantation. The present pilot study demonstrates a novel way of maximising establishment of *Amphibolis antarctica* seedlings. Seedlings were planted and tethered using an innovative spiral peg, in a bare sand area adjacent to a mature *Amphibolis* meadow in Shoalwater Bay, Western Australia. Survival and growth were monitored over two years. After two years, 29.4% of seedlings survived into well-established mature plants. By enhancing establishment success of seedlings it is anticipated that more rapid and sustainable rehabilitation of *Amphibolis* seedlings will be possible.

Keywords: Amphibolis antarctica; Anchoring method; Seagrass; Seedlings; Transplantation

Introduction

The past century has seen major losses of seagrass ecosystems around the world, resulting from a range of human-induced and natural phenomena; 29% of global seagrass area has been lost since 1879 and losses have accelerated to 110 km^2 per year since 1980 (Waycott et al., 2009). Seagrasses have been ranked as one of the most ecologically and economically valuable biological systems on earth, with key roles as ecosystem engineers, protecting coastlines from erosion; as nursery areas for commercially and recreationally important species; in nutrient cycling; and in sequestering atmospheric carbon for thousands of years (Barbier et al., 2011 and Fourqurean et al., 2012).

These ecosystem services are extremely valuable with estimates for the value of seagrass habitats ranging from US$12,635 to $25,270 ha^{-1} yr^{-1} (Lothian, 1999); a more recent study has placed the value of seagrass habitats at US$34,000 ha^{-1} yr^{-1} (Short et al., 2011). Growing awareness of the importance of these services has resulted in increased government regulation of coastal development to improve protection of seagrass ecosystems. An important tool for regulators is the use of mitigation for seagrass losses by developers; this includes environmental offsets such as rehabilitation programmes.

Substantial losses of *Amphibolis antarctica* meadows have occurred off Adelaide and natural recovery of the species in the region is poor. Rivers et al. (2011) showed that survival of *A. antarctica* seedlings planted into sand and onto natural seagrass rhizome mat was poor (approximately 6%), highlighting the limited extent of natural recruitment. Large scale rehabilitation efforts have shown only moderate success (Bryars, 2008), and alternative methods to improve recruitment of the viviparous seedlings on a larger scale have been examined. Recent studies showed that hessian bags appear to be very efficient in capturing seedlings (Seddon et al., 2004, Wear et al., 2006 and Wear et al., 2010); however while recruitment of seedlings onto these artificial structures is high, survival and retention of seedlings onto these structures remain a problem (Bryars, 2008, Seddon et al., 2004, Wear et al., 2006 and Wear et al., 2010). In a small scale study only 10% of *Amphibolis* recruits were retained after eight months (Wear et al., 2010).

Seagrass rehabilitation to date has mainly involved transplanting rhizomes and shoots from healthy existing meadows to non-vegetated coastal sediment (Fishman et al., 2004, Fonseca et al., 1998, Paling et al., 2009, Treat and Lewis, 2006 and Verduin et al., 2012); the use of seeds and seedlings has been problematic in many instances, particularly for slower growing temperate seagrasses in Australia (Kirkman, 1998 and Wear et al., 2010). Transplant projects incorporating seedlings have been relatively rare in Australia; however in the move to sustainable, non-destructive rehabilitation methods, the use of seeds and seedlings is vital to enhancing rehabilitation efforts. For instance *Zostera marina* seed trials have been very successful in the US in uni-directional flow regimes (Orth et al., 2012). Alternative sources of planting units (e.g., seeds or seedlings) will need to be explored to guarantee the viability of seagrass restoration efforts. Use of seeds and seedlings will also maximise genetic diversity of transplanted meadows (Sinclair et al., 2013), improving resistance to environmental stresses. The present study provides the first steps towards alternative techniques maximising seedling establishment success.

In southern and south-Western Australia, viviparous seedling-bearing seagrasses*Amphibolis antarctica* and *A. griffithii* are abundant, forming extensive mono-specific and mixed species meadows. This study focuses on *A. antarctica* because of significant recent losses of this species. A few studies have trialled transplanting *Posidonia* and *Amphibolis* seedlings with varying degrees of success (Kirkman, 1998 and Wear et al., 2006). Several media such as seagrass matte fibre and hessian bags were tested (Wear et al., 2010 and references therein). In Western Australia several attempts to rehabilitate *Amphibolis antarctica* have been undertaken, resulting in the realisation that recruitment is less of a problem than keeping the seedlings in place (Kirkman, 1998, Verduin and van Keulen, 2012 and Verduin et al., 2010). Rivers et al. (2011) suggested that the high loss of seedlings in natural environments is driven by hydrodynamic forcing.

Survival and establishment of *A. antarctica* seedlings are dependent on entanglement of a specialised structure, the comb anchor (Fig. 1), with fibrous material such as seagrass roots and rhizome fibres found in seagrass meadows (Kuo and Kirkman, 1990). The energy needed to displace entangled seedlings is generally influenced by the strength of the material to which it was attached, whereas the force to dislodge a seedling from sand is influenced only by the weight of sand covering the comb anchor (Rivers et al., 2011). This supports observations of poor seedling transplant success into bare sand (Irving et al., 2010 and Kirkman, 1998).

Finding an effective solution to the problem of seagrass seed-ling dislodgment and maximising genetic diversity of transplanted material are critical to sustainable seagrass rehabilitation. The current study presents a novel anchoring technique to address a key problem in the establishment of seagrass seedlings, with a focus on *Amphibolis antarctica*.

Methods

Amphibolis antarctica seedlings were collected in Shoalwater Bay and transplanted to a nearby bare sand area. Seedlings were found attached to *Posidonia australis* fibrous material along an exposed scarp in Shoalwater Bay (S 32° 16.394′, E 115° 41.566′) shortly after release of the seedlings from the parent plant. Individual recruited seedlings (n = 85) were secured to a purpose-designed metal wire peg with a spiral structure to hold the seedling (Fig. 1). The seedlings were planted immediately following collection into a bare sand area close to an existing *Amphibolis antarctica* meadow in Shoalwater Bay; the seedlings were kept submerged at all times. Seedlings were planted 1.5 m away from existing plants thus avoiding physical contact with adult plants. Transplanted seedlings were monitored for establishment and growth at 3, 12 and 24 months.

Results

After three months 75.5% of the transplanted seedlings were retained. One year after planting 29.4% of all seedlings survived (Table 1). The remaining seedlings grew from a mean length of 8.2 ± 0.11 cm at transplantation to 20.3 ± 0.68 cm. In June 2012, two years after initial planting, there had been no further loss of seedlings, i.e. 29.4% survival; these had grown to a mean shoot length of 46.11 ± 1.11 cm (Table 1). The spiral pegs successfully remained fixed to the seabed; over time an encrusting growth developed on the pegs which did not seem to restrict the growth of the seedlings. The loss of seedlings in this experiment was most likely associated with the sediment dynamics at the recipient site, with burial as the major cause.

Discussion

The spiral pegs proved an effective means of stabilising and retaining seedlings, compared with previous attempts to transplant *Amphibolis* seedlings. Although losses in the first year after transplantation were significant, sufficient seedlings survived to confirm the beneficial role of the spiral pegs in long-term establishment of the seedlings. Several wire pegs were encrusted with calcareous algae, which may have restricted growth of some of the seedlings; however good growth of the seedlings was observed overall, with consistent extension of surviving seedlings over the two years of the experiment.

Seagrass shoots exposed to orbital flow from ocean swell rapidly move backward and forward and can change orientation over periods of seconds (Backhaus and Verduin, 2008 and Koch et al., 2006). Physical contact with nearby adult plants has been shown to cause seedling dislodgement and removal by abrasion (Irving and Connell, 2006 and Rivers et al., 2011). The choice of transplant site, therefore, is as important as the quality of the plant material used for rehabilitation (Verduin et al., 2012). Sediment in the high energy environments usually favoured by *Amphibolis* meadows is generally fluid in nature. While seedling loss has previously been attributed to erosion of sediment and therefore dislodgement of the seedlings, burial was identified as the major cause of loss of the seedlings in this experiment, reflecting the increased tethering effect of the spiral pegs.

Field observations of *Amphibolis* seedling settling behaviour in Western Australia show that naturally recruited seedlings are generally upright to an angle of 45° and have freedom of movement even when attached to the fibrous rhizomes of *Posidonia* sprigs. The new attachment design allows for this movement and yet holds the seedlings in place. Our pilot study showed a 29.4% survival over two years in a very dynamic coastal area, a significant improvement compared to similar small scale studies in South Australia where only 10% of recruits were retained after eight months (Wear et al., 2010), and even less for untethered seedlings in south-Western Australia (Rivers et al., 2011).

Survival is often chosen as a measure of success; however, it is also vital to monitor growth. In the present study the 29.4% surviving *Amphibolis* seedlings had an average of 30 cm growth over two years (Table 1). Despite seedling burial and encrusting growth on the anchoring pegs, 29.4% survival of the seedlings and good growth after two years suggests that use of anchoring pegs may be a viable means of enhancing seedling establishment.

The aim of the current study was to establish the effectiveness of the spiral pegs to hold the seedlings in place, yet allow for natural movement with water flow. *Amphibolis* generally grows in high energy environments; the present study was conducted in a fairly typical *Amphibolis* habitat subject to significant orbital wave energy. Further studies and larger scale rehabilitation attempts will examine a broader range of *Amphibolis* habitats.

Recruitment of *A. antarctica* seedlings within and adjacent to existing seagrass meadows may also contribute to genetic diversity in this system. A recent study on a *Posidonia* *australis* rehabilitation site showed that by transplanting adult plants to a new area high genetic diversity in the donor site was captured in the donor material used for the restored meadow (Sinclair et al., 2013).

Paucity of establishing seedlings at a site may be caused by recruitment constraints such as hydrodynamic conditions preventing seedlings reaching a suitable site (Rivers et al., 2011), or viability of seedlings (Eriksson, 1989 and Eriksson and Ehrlen, 1992). As there is a move to sustainable use of non-destructive rehabilitation methods, particularly using seeds and seedlings, the research presented here is vital to enhancing natural rehabilitation efforts. The refining of field techniques (e.g. through improved local site selection for transplants) can improve the success of restoring *Amphibolis antarctica* meadows, at least on a small scale. Areas adjacent to Cockburn Sound have healthy seagrass meadows that produce very large numbers of seedlings annually. We suggest that future natural recruitment through dispersal of seedlings may help to ensure the long-term viability of restored seagrass meadows initially established with transplants of *Amphibolis antarctica* in southwest Western Australia.

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Fig. 1. A) Viviparous *Amphibolis antarctica* seedling with the unique comb anchor mechanism (circled). B) Spiral peg used to anchor *Amphibolis* seedling in the transplantation trial.

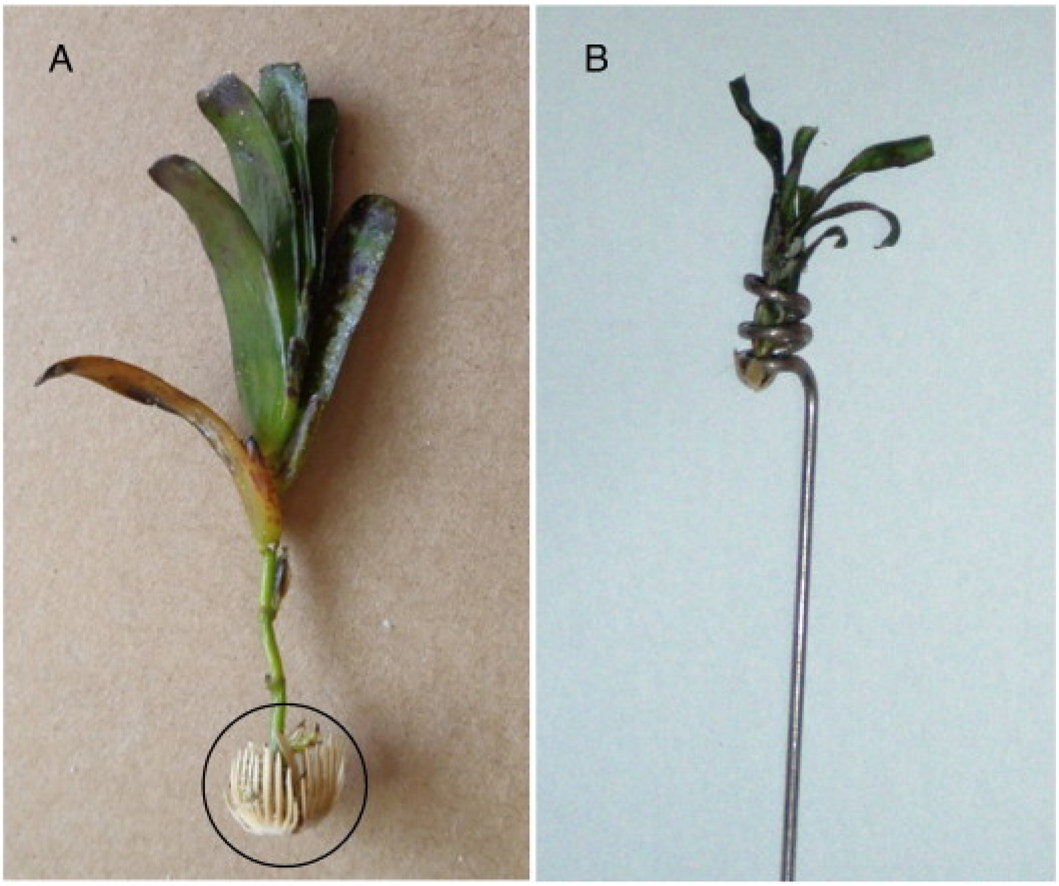

Table 1. *Amphibolis* seedling survival and growth at 3, 12 and 24 months.

Time (Months)	0	3	12	24
Mean seedling length ± SE	8.19 ± 0.11	11.42 ± 0.73	20.31 ± 0.68	46.11 ± 1.11
Seedling survival %		75	29.4	9.4